Incorporation of Weather Forecasting in Integrated, Biological-Chemical Management of *Botrytis cinerea*

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ABSTRACT

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A strategy for integrated biological and chemical control of *Botrytis cinerea* in nonheated greenhouse vegetables was developed. The biocontrol agent used was a commercial preparation developed from an isolate of *Trichoderma harzianum*, T39 (Trichodex). Decisions concerning whether to spray the biocontrol agent or a fungicide were made based on a weather-based disease warning system. The integrated strategy (BOTMAN [short for Botrytis manager]) was implemented as follows: when slow or no disease progress was expected, no spraying was needed; when an outbreak of epidemics was expected, use of a chemical fungicide was recommended; in all other cases, application of *T. harzianum* T39 was recommended. Future weather information (a 4-day weather forecast pro-

vided by the Israel Weather Forecast Service) was more useful for disease warnings than immediate past weather. The integrated strategy was compared with weekly applications of fungicide in 11 experiments conducted over 3 years in greenhouse-grown tomato and cucumber. Disease reduction in the integrated strategy ($63.9 \pm 3.0\%$) did not differ significantly ($P < 0.05$) from the fungicide-only treatment ($70.1 \pm 3.6\%$). The number of fungicide sprays in the integrated strategy ranged from 2 to 7 (mean 4.2) compared to 7 to 13 (mean 10.5) in the fungicide treatment. The integrated strategy averaged 5.9 sprays of *T. harzianum* T39. For the integrated strategy, one treatment omitted use of *T. harzianum* T39 to estimate the contribution of this agent to disease control. Disease reduction in that treatment ($49.1 \pm 4.8\%$) was significantly ($P < 0.05$) inferior to the combined chemical and biological strategy, indicating that the *T. harzianum* T39 sprays had a measurable effect on disease control.

Botrytis cinerea Pers.:Fr., the causal agent of gray mold, attacks leaves, stems, flowers, and fruits of various vegetable crops grown in greenhouses. In nonheated greenhouses, the pathogen primarily infects leaves (e.g., tomato) or fruits (e.g., cucumber and tomato), but lesions can expand to other tissues (stems). In heated greenhouses, the occurrence of leaf and fruit infections are limited, and infections of pruning wounds are more common. Stems girdled by disease can result in substantial yield losses. Gray mold epidemics are a serious problem in vegetable greenhouses and are often the reason for finishing a crop earlier than planned (5,21,22,31,34). In general, growers use fungicides to control gray mold diseases. Both spraying of fungicides and application directly to infected wounds are practiced. However, the frequent development of *B. cinerea* populations resistant to common fungicides and the desire to reduce pesticide use have led to efforts to develop alternative measures for disease management, such as biological control.

Several biocontrol agents are reportedly effective against *B. cinerea* (10), among which are isolates of the filamentous fungus *Trichoderma harzianum* Rifai. A commercial preparation developed from isolate T39 of *T. harzianum* (Trichodex) is registered for agricultural use in Israel and other countries. In a recent analysis of 64 experiments carried out in nonheated vegetable greenhouses during 1988 to 1993, *T. harzianum* T39 when applied alone was as effective as the standard chemical treatment in 70% of the trials. In some experiments (20%), however, disease suppression achieved by *T. harzianum* T39 was significantly inferior to that of the standard chemical treatment, and in a few experiments (10%), *T. harzianum* T39 efficacy was insufficient. Inconsistency and variability in control efficacy among biocontrol agents are common (7). The reasons for this are not well understood, but the phenom-

enon hinders the implementation of biocontrol agents as stand-alone measures for disease management. One way to improve the reliability of biocontrol agents is to integrate them with chemical fungicides.

Integration of biological and chemical controls has been investigated in some studies. Alternation of *T. harzianum* T39 with chemical fungicides on a weekly basis resulted in disease suppression that was as effective as that achieved by applying the fungicide alone and was more consistent than *T. harzianum* T39 applied alone (8,15,16,27). Although the alternating treatment was effective and provided a 50% reduction in the number of chemical sprays, it had an important drawback in that the biocontrol agent was applied at predetermined, fixed intervals. *T. harzianum* T39 is a living organism that is affected by the environment. Accordingly, it is possible that under certain conditions application of this agent would provide significant control but that under other conditions a fungicide would be more effective. We hypothesized that integration of chemical and biocontrol agents, such that the biocontrol agent would be applied when it was likely to be effective, would enable a further reduction in the number of chemical sprays while achieving the same level of disease control. To achieve this goal, the biology of both the pathogen and the biocontrol agent were considered.

The ecological requirements of *B. cinerea* and *T. harzianum* T39 differ to some extent. In general, *Botrytis* epidemics occur under cool, humid conditions that favor infection and also may predispose the host to susceptibility (20). High relative humidity (RH) in the greenhouse and free moisture on plant surfaces are required for conidia of *B. cinerea* to germinate, produce a germ tube, and infect the host (1,2,4,6,20,26,29,35). Optimum temperatures for spore germination and infection are between 9 and 21°C, but they can occur at 2°C and >25°C (6,20,26,29). After penetration, the mycelium is relatively protected by the host tissue, and temperature and RH requirements are less stringent (1,29). Sporulation of *B. cinerea* on infected tissues occurs at a wide range of temperatures, in-

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cluding low temperatures in cold-storage facilities. Dry conidia of *B. cinerea* are released when the RH drops (18). Due to *B. cinerea*'s strict requirement of free moisture during spore germination and penetration, progress of *Botrytis* epidemics in nonheated greenhouses is characterized by an outbreak of the disease and not by the common logistic increase in infected host tissue (12,13,35). The duration of these outbreaks may range from several days to a few weeks. When conditions are suboptimal for the pathogen, disease progresses at a moderate rate; when conditions are unfavorable, disease progresses slowly or not at all.

Conidia of *T. harzianum* T39 survive best at high temperatures (20 to 26°C) and RH (80 to 97%) (9), providing the most effective control under conditions of low vapor pressure deficit, but not when the air is saturated or dew is deposited on the foliage, and at 20 to 26°C (17,27). Comparing the ecological requirements of *B. cinerea* and *T. harzianum* T39 suggests that *T. harzianum* T39 would be most effective when conditions are suboptimal for *Botrytis* development. When conditions are highly conducive to the pathogen, the efficacy of *T. harzianum* T39 is expected to be low. In this study, we describe concepts for integration of biological and chemical controls of *B. cinerea* in nonheated greenhouse vegetables in relation to their ecological requirements. An integrated control strategy (BOTMAN [short for Botrytis manager]) was developed and examined in 11 experiments under diverse conditions over 3 years in commercial, nonheated greenhouse-grown tomato and cucumber plants in Israel.

MATERIALS AND METHODS

Development of an integrated control strategy. Initial efforts were devoted to developing a system to predict the relative favorability of the environment for infection by *B. cinerea*. This tool was used to determine which control method (biological or fungicide) would be most appropriate and served as the basis for the integration of control methods. The suitability of the environment to *B. cinerea* infection was quantified on the basis of published information (11,12,14,20–22). Both past weather in the preceding 7 days and a 4-day forecast of future weather were considered as sources of data to quantify the favorability of the environment for disease development. For past weather, a warning system developed recently by Yunis et al. (35) for greenhouse cucumber plants was implemented. This system predicts outbreaks of gray mold when, on a weekly average, the wetting period of the foliage exceeds 7 h/day and the duration of temperatures between 9 and 21°C during the night (1800 to 0800 h) exceeds 9.5 h/day. Moderate development of disease is expected when only one of these criteria has been met. Disease is expected to develop slowly or not at all when neither criteria is met.

Measurements of air and leaf temperatures and RH in the greenhouse enabled us to quantify the suitability of past weather for *B. cinerea* development. The data were recorded hourly by C21 X data loggers (Campbell Scientific, Inc., Logan, UT). Air and leaf temperatures were measured with copper-constantan thermocouples, and RH was measured with an electro-humidity sensor. Ten thermocouples for measuring air and leaf temperatures and five RH sensors were placed in each greenhouse at a height of 1 to 1.5 m inside the canopy, with shields facing south to protect the sensors from intense radiation. Thermocouples for measuring leaf temperature were attached to the lower portions of leaves. Deposition of dew on the plant canopy was estimated by calculating the dew-point temperature. The foliage was considered wet whenever the leaf temperature was below the dew-point temperature (34). On the basis of these records, the daily duration of leaf wetness and temperature between 9 and 21°C during the night were calculated.

For future weather, 4-day weather forecasts provided by the Israel Weather Forecast Service were used. Forecasts were converted to a disease risk index by an approach described by Madden and Ellis (25) and Kelly (23). For each environmental parameter

thought to be conclusive for gray mold development, an empirical severity value was assigned to a portion of the parameter range (Table 1). The severity values reflected the relative importance of each parameter in intensifying the disease. Parameters, potential ranges, and severity values were based on published results (13, 14,35). The sum of all severity values resulted in the risk index for gray mold. Empirical thresholds for the risk index were determined to distinguish situations in which (i) severe disease outbreaks were expected, (ii) moderate disease progress was expected, or (iii) slow or no disease progress was expected. Using results of previous greenhouse experiments, different scenarios of weather and disease progress were examined, and the thresholds for the risk value were defined as >4.6, 2.5 to 4.5, and <2.4 for the three disease expectations, respectively.

A special weather forecast for BOTMAN was prepared by the agricultural meteorologist at the Israeli Weather Forecast Service. The forecast included the following parameters for each of the 4 days in the forecast: maximal and minimal temperatures, rain (number of days of occurrence and quantity), cloudiness (5/8 sky cover for >6 h during the day), and occurrence of hot, dry weather. A forecast was prepared for each experimental site and transmitted by fax. Weather parameters were analyzed according to the criteria listed in Table 1 and used to determine the suitability of future weather for infection by the pathogen and for biological control by the agent.

The weather-based indices of disease risk provided a framework for integration of biological and chemical methods for gray mold control. Decisions concerning whether to apply a biocontrol agent or a fungicide were made before each spray according to the value produced by the disease risk indices. The rules for decision making were (i) do not spray when slow or no disease progress was expected, (ii) spray *T. harzianum* T39 when moderate disease was expected, and (iii) spray a chemical fungicide when disease outbreak was expected.

Evaluation of the integrated control strategy. Evaluation of the integrated strategy was done in two steps. First, we examined

TABLE 1. Parameters, ranges, and severity values used in the gray mold warning system developed based on weather forecasts for 4-day intervals^z

Parameter	Parameter range	Assigned severity value
Rain quantity (mm/day)	0.0–0.4	0.0
	0.5–5.0	0.2
	5.1–10.0	0.4
	10.1–20.0	0.6
	20.1–30.0	1.0
	>30	1.2
No. of rainy days (>0.5 mm/day)	0	0.0
	1	0.2
	2	0.4
	3	0.8
	4	1.1
Maximum temperature (C)	<9	0.1
	10–21	0.3
	22–26	0.1
	<26	0.0
Minimum temperature (C)	<5	0.1
	6–9	0.2
	10–21	0.3
	>21	0.0
No. of days with 5/8 cloud cover (>6 h during the day)	0	0.0
	1–2	0.2
	3–4	0.5
No. of days with hot, dry weather (<25% RH for more than 4 h)	0	1.0
	1–2	0.5
	3–4	0

^z The sum of all severity values resulted in the risk index for gray mold. Severe disease outbreaks are expected when the risk index is >4.6; moderate disease is expected when the risk index is between 2.5 and 4.5; and slow or no disease progress is expected when the risk index is <2.4.

whether environmental data based on past weather, future weather, or both were most useful for decision making within the strategy. Next, performance of BOTMAN was compared to a conventional fungicide-only control program, with four experiments on tomato (T1 to T4) and seven experiments on cucumber (C1 to C7). Experiment T1 was conducted in 1994, experiments T2 and C1 through C3 were conducted in 1995, and experiments T3 through T4 and C4 through C7 were conducted in 1996. All experiments were done in commercial greenhouses located in the central and southern coastal plain of Israel. Tomato (*Lycopersicon esculentum* Mill. 'F175,' 'F144,' and '198') and cucumber (*Cucumis sativus* L. 'Carim,' 'Alaska,' 'Muhasan,' and 'Dina') plants were planted during September of each year. All cultivars were susceptible to *B. cinerea*. Polyethylene-covered greenhouses were not heated or ventilated electrically but had side vents that were opened for 5 to 8 h daily when weather permitted. Plants were spaced 0.5 m apart within rows, with 1.25 m between rows. Plants were grown in a high-wire cropping system and maintained according to the recommendations of the extension service in the region. No fungicides for the control of gray mold were applied, except those outlined in the experimental design (Table 2 and Figs. 1 through 3).

Experimental design. Experiments were arranged in a randomized block, with five replicates per treatment. Each plot was one row 8 to 12 m long (15 to 25 plants). In most experiments, the plots were separated from each other by guard rows. All experiments shared four similar treatments and an additional one to three treatments that were included to address specific questions. The four common treatments were (i) untreated control; (ii) weekly fungicide only, in which the commercial practices for management of *B. cinerea* were followed: fungicides from different chemical groups and with different modes of action were alternated in a 7-day interval sequence (in 1996 *T. harzianum* T39 was included occasionally in the sequence of sprays because Trichodex was registered and used commercially as any other chemical com-

pound); (iii) BOTMAN, in which fungicides and *T. harzianum* T39 were implemented in a 7-day schedule according to the decision-making rules developed for BOTMAN; and (iv) fungicide only applied according to BOTMAN, in which fungicide applications coincided with the time of application in treatment three. This treatment was included to identify the added contribution of *T. harzianum* T39 sprays in the BOTMAN treatment. In 1994 and 1995, the tomato experiments included two additional treatments. In the first, BOTMAN sprays were based on the past weather warning system (35), and in the second, BOTMAN sprays were based on both past and future weather. For the latter treatment, a fungicide was sprayed if either system predicted a severe outbreak, and *T. harzianum* T39 was applied if the systems predicted moderate disease progress. In addition, the 1994 and 1995 tomato experiments and the 1995 cucumber experiments included a *T. harzianum* T39-only treatment applied at 7-day intervals.

Because the decision-making procedure has not yet been computerized, BOTMAN was operated manually. A decision regarding which control agent (i.e., chemical or biological) should be applied was made before each spray based on the concepts listed above. All the necessary information was collected and analyzed just before each spray, and instructions were delivered by phone to the sprayers.

Natural epidemics of *B. cinerea* developed in the greenhouses during the winter seasons. Spraying was initiated in all experiments when the first gray mold symptoms were observed. This occurred during early December in the 1995 tomato experiment and during early- to mid-January in the rest of the experiments. Fungicides and *T. harzianum* T39 were applied with a backpack sprayer adjusted to 5 atm of pressure or by a gun sprayer. Care was taken to avoid drift to adjacent plots. Spray volume was calibrated to 1,000 to 1,500 liters of water per ha. The fungicides used were as follows: iprodione (0.5 g a.i./liter of Rovral 50WP); vinclozolin (0.5 g a.i./liter of Ronilan 50WP); chlorothalonil (1.75 g

TABLE 2. Incidence of gray mold on fruits and stems of greenhouse-grown cucumber plants treated with chemical fungicides and a biocontrol agent (*Trichoderma harzianum* T39) in five experiments and number of sprays applied in each treatment^w

Cucumber experiment	Treatment ^x	Spraying sequence ^y										No. of sprays		Infections/plant ^z	
												Fungicide	T39	Fruits	Stems
C2	Untreated control	...	...	...	...	...	...	...	...	...	...	0	0	13.6 a	4.1 a
	Weekly fungicide	PF	I	PF	I	PF	CD	TD	P	PF	I	10	0	3.5 b	0.9 c
	T39	Th	Th	Th	Th	Th	Th	Th	Th	Th	Th	0	10	5.8 b	2.3 b
	BOTMAN	Th	Th	Th	I	PF	Th	TD	P	Th	Th	4	6	3.5 b	0.9 c
	Fungicide-BOTMAN	...	...	...	I	PF	...	TD	P	...	...	4	0	4.9 b	2.1 b
C3	Untreated control	...	...	...	...	...	...	...	...	...	...	0	0	29.9 a	2.7 a
	Weekly fungicide	PF	I	PF	P	PF	CD	P	TD	I	CD	10	0	15.6 c	0.8 b
	T39	Th	Th	Th	Th	Th	Th	Th	Th	Th	Th	0	10	18.8 bc	1.4 ab
	BOTMAN	Th	Th	Th	P	PF	Th	P	TD	Th	Th	4	6	20.0 bc	1.0 b
	Fungicide-BOTMAN	...	...	...	P	PF	...	P	TD	...	...	4	0	22.4 b	1.5 ab
C5	Untreated control	...	...	...	...	...	...	...	...	...	...	0	0	7.7 a	6.5 a
	Weekly fungicide	TD	P	Th	I	C	TD	Th	I	Th	P	9	3	3.0 b	2.0 c
	BOTMAN	Th	P	Th	Th	C	Th	Th	I	P	Th	4	8	3.3 b	2.3 c
	Fungicide-BOTMAN	...	P	...	...	C	...	...	I	P	...	4	0	4.3 b	3.5 b
C6	Untreated control	...	...	...	...	...	...	...	...	...	...	0	0	12.8 a	1.8 a
	Weekly fungicide	Th	P	I	F	CD	Th	P	I	CD	P	8	2	5.5 b	0.3 b
	BOTMAN	Th	P	I	Th	CD	Th	Th	P	P	Th	5	5	7.1 b	0.2 b
	Fungicide-BOTMAN	...	P	I	...	CD	...	...	P	P	...	5	0	8.0 b	0.4 b
C7	Untreated control	...	...	...	...	...	...	...	...	...	...	0	0	4.6 a	2.8 a
	Weekly fungicide	I	P	Th	F	CD	I	P	Th	CD	P	10	2	1.8 b	1.0 c
	BOTMAN	Th	P	Th	Th	I	Th	P	CD	P	Th	5	7	2.1 b	1.3 bc
	Fungicide-BOTMAN	...	P	...	...	I	...	P	CD	P	...	5	0	2.5 b	1.8 a-c

^w Mold incidence on fruits is the cumulative number of infected fruits collected throughout the entire season. Incidence on stems was recorded on the last assessment date.

^x In the BOTMAN (Botrytis manager) treatment, fungicides and *T. harzianum* T39 were sprayed in a 7-day schedule based on future weather forecasts. In the fungicide-BOTMAN treatment, fungicide applications coincided with the application times in the BOTMAN treatment.

^y Spraying was initiated when the first symptoms caused by *B. cinerea* were observed. Compounds: C = chlorothalonil, F = fenbuconazole, I = iprodione, P = pyrimethanil, V = vinclozolin, CD = carbendazim + diethofencarb, PF = prochloraz + folpet, TD = tebuconazole + dichlofluanid, Th = *T. harzianum* T39 (Trichodex), and ... = not applied.

^z Within each column and experiment, numbers followed by common letters do not differ significantly according to Fisher's protected LSD test ($P \leq 0.05$).

a.i./liter of Bravo 50SC); fenbuconazole (0.1 g a.i./liter of Indar 5EC); and pyrimethanil (0.75 g a.i./liter of Mythos 30SL). In addition, the following fungicide mixtures were applied: carbendazim + diethofencarb (0.25 + 0.25 g a.i./liter of Resec 25 + 25WP); pro-

chloraz + folpet (0.45 + 1.8 g a.i./liter of Mirage F 15 + 60WP); and tebuconazole + dichlofluanid (0.25 + 0.62 g a.i./liter of Silvasure 10 + 25WP). The rate of the biocontrol agent *T. harzianum* T39 was 0.5 g a.i./liter of Trichodex 25SP. The number of fungicide

Fig. 1. Incidence of gray mold on leaves and stems of greenhouse-grown tomato plants treated with fungicides and a biocontrol agent (*Trichoderma harzianum* T39) in two experiments. **A**, Experiment T1 in 1994; **B**, experiment T2 in 1995. The occurrence and quantity of rain are indicated in the upper graph by histograms. In the BOTMAN (Botrytis manager) treatment, fungicide and T39 sprays were implemented in a 7-day schedule based on forecasts of future weather; in the BOTMAN-past treatment, fungicide and T39 sprays were implemented based on past weather; in the BOTMAN-past + future treatment, fungicide and T39 sprays were implemented based on both. In the fungicide-BOTMAN treatment, fungicide applications coincided with the time of application in the BOTMAN treatment. The time of spraying and type of compound applied are indicated above each graph: C = chlorothalonil, F = fenbuconazole, I = iprodione, P = pyrimethanil, V = vinclozolin, PF = prochloraz + folpet, TD = tebuconazole + dichlofluanid, and Th = *T. harzianum* T39. Incidence of stem infection was recorded on the last assessment date. For leaf infection, vertical bars represent the LSD for each sampling date; for stem infection, data in bars with common letters do not differ significantly according to Fisher's protected LSD test ($P \leq 0.05$).

and *T. harzianum* T39 sprays and the time of application are shown in Figures 1 through 3 and Table 2.

For use in experiments, fungicides were divided into groups according to their mode of action. If a chemical fungicide was to be applied according to BOTMAN, the choice of the specific compound was made based on several criteria: (i) the relative efficacy of the fungicide (e.g., the most effective material was used with the highest severity index); and (ii) the frequency of resistance of the *B. cinerea* strain in the greenhouse (i.e., when >40% of the *B. cinerea* population was resistant to a fungicide, use of this chemical was stopped). The frequency of fungicide resistance in *B. cinerea* populations was evaluated in each greenhouse two or three times during the season (January through March) as described previously (12). Additional criteria that influenced the choice of fungicide included the history of chemical materials used during the season (an attempt was made to alternate among fungicides with different modes of action or to use fungicides with broad-spectrum activity) and the severity of *B. cinerea* infection in the greenhouse (e.g., when disease was severe, a more effective material was chosen).

Disease assessment. Disease incidence was assessed every 7 to 14 days on 7 to 10 plants in the center of each experimental plot.

Fig. 2. Gray mold progress curves on greenhouse-grown tomato plants treated with fungicides and a biocontrol agent (*Trichoderma harzianum* T39) in two experiments in 1996: **A**, experiment T3; **B**, experiment T4. In the BOTMAN (Botrytis manager) treatment, fungicides and T39 were implemented in a 7-day schedule based on future weather forecasts. In the fungicide-BOTMAN treatment, fungicide applications coincided with the time of application in the BOTMAN treatment. The time of spraying and type of compound applied are indicated above each graph: I = iprodione, P = pyrimethanil, CD = carbendazim + diethofencarb, TD = tebuconazole + dichlofluanid, and Th = *T. harzianum* T39. For each sampling date, vertical bars represent the LSD according to Fisher's protected LSD test ($P \leq 0.05$).

For cucumber, the number of infected fruits and stem lesions was counted. Diseased cucumber fruits were removed after each assessment. For data analysis, the cumulative number of infected fruits was calculated. For tomato, the number of infected leaves and stem lesions was counted on each plant. Diseased tomato leaves were not removed. In two tomato experiments in 1996, the proportion of dead plants per plot was determined. Results were subjected to analysis of variance, and Fisher's protected LSD test was applied at $P \leq 0.05$. Disease reduction was calculated according to the formula $(a - b)/a \times 100$, where a = disease incidence in the untreated control and b = disease incidence in the relevant treatment. Disease reduction was calculated for all categories of disease incidence: number of infected leaves, fruits, and stems per plant and proportion of dead plants.

RESULTS

In each experiment, the onset of gray mold coincided closely with the occurrence of cold, rainy weather. After its appearance, disease continued to develop for 2 to 3 months and slowed when the rains stopped and temperatures rose during early spring. During disease development, there were several periods of rapid disease increase that were preceded by rain events. For example, in 1994, three periods of disease increase were observed in the untreated tomato plots: 17 to 20, 27 to 38, and 59 to 65 days after 1 January (Fig. 1A). Periods of rapid disease increase also were apparent in other experiments (Figs. 1B, 2, and 3).

Disease intensity varied among experiments (Figs. 1 through 3 and Table 2). The most severe epidemic in tomato occurred in 1996 (experiment T3), during which 71.4% of the plants in untreated plots were killed by gray mold (Fig. 2A). The most severe epidemic of the cucumber experiments was observed in 1995 (experiment C3), during which 29.9 infected fruits per plant were diseased (Table 2). RH and leaf wetness duration were substantially higher in these greenhouses compared to the others.

The 7-day-interval fungicide treatment resulted in 7 to 13 weekly applications per experiment (mean = 10.5). This treatment, in general, was very effective and reduced disease incidence in all experiments significantly compared with incidence in the untreated plots (Figs. 1 through 3 and Table 2). Disease reduction in the standard fungicide treatment in all experiments was $70.1 \pm 3.6\%$ (mean of 20 comparisons of all categories of disease incidence plus/minus standard error [SE]). The routine tests for identification of *B. cinerea* populations resistant to common fungicides in the greenhouses revealed strains resistant to dicarboximide (iprodione and vinclozolin) and ergosterol biosynthesis inhibitors (tebuconazole and fenbuconazole) fungicides and to diethofencarb and carbendazim. After detection of resistance, the relevant compounds were excluded from further use.

The efficacy of *T. harzianum* T39 applied alone varied among two tomato experiments in 1994 and 1995 and three 1995 cucumber experiments. In some cases, the biocontrol agent was as effective as the chemical treatment (e.g., experiment C2; Table 2); in others, it significantly reduced disease incidence compared with the untreated control but was inferior to the standard fungicide treatment (e.g., experiment T1; Fig. 1A). In a few cases, *T. harzianum* T39 was completely ineffective (e.g., fruit infection, experiment C1; Fig. 3A). Average disease reduction in the *T. harzianum* T39 treatment was $41.2 \pm 3.7\%$ versus $79.7 \pm 4.5\%$ (mean of 10 comparisons plus/minus SE) in the standard fungicide treatment (for the same experiments).

The type of weather information (i.e., past, future, or both) needed for BOTMAN was tested in tomato experiments in 1994 and 1995. In 1994 the number of fungicide sprays applied within the sequence of *T. harzianum* T39 sprays was seven, eight, and three for BOTMAN decision making based on past, future, or past and future weather, respectively. In 1995 four, three, and one fungicide applications were made for the same treatments, respectively. Al-

though leaf and stem infections were significantly lower in all three BOTMAN treatments than in the untreated control, the treatment based on future weather was the most effective: disease reduction was $75.1 \pm 2.1\%$ versus $58.5 \pm 3.6\%$, $55.1 \pm 4.9\%$, and $83.3 \pm 4.2\%$ (mean of four comparisons plus/minus SE) in the BOTMAN future weather, BOTMAN past weather, BOTMAN past and future weather, and weekly fungicide only treatments, respectively. We concluded that for decision making, future weather is impera-

tive; thus, subsequent BOTMAN experiments were based on future weather.

The weather forecasts provided by the Israel Weather Forecast Service were reasonably accurate (Table 3). For two experimental sites, the central and southern coastal plain, there were 28 rain events over 104 days, of which the occurrence of 22 (78.6%) was predicted within a 4-day forecast period. Dry days were forecast correctly for 70 of 76 days (92.1%). In 87 of the 104 daily fore-

Fig. 3. Incidence of gray mold on fruits and stems of greenhouse-grown cucumber plants treated with chemical fungicides and a biocontrol agent (*Trichoderma harzianum* T39) in two experiments. **A**, Experiment C1 in 1995; **B**, experiment C4 in 1996. In the BOTMAN (Botrytis manager) treatment, fungicides and T39 were implemented in a 7-day schedule based on future weather. In the fungicide-BOTMAN treatment, fungicide applications coincided with the time of application in the BOTMAN treatment. The time of spraying and type of compound applied are indicated above each graph: I = iprodione, P = pyrimethanil, CD = carbendazim + diethofencarb, TD = tebuconazole + dichlofluanid, and Th = *T. harzianum* T39. Incidence of stem infection was recorded on the last assessment date. For fruit infection, vertical bars represent the LSD for each sampling date; for stem infection, the data bars with common letters do not differ significantly according to Fisher's protected LSD test ($P \leq 0.05$).

casts (83.6%), the quantity of rain was predicted accurately. Forecasts for the other meteorological parameters in 1996 and for previous years were slightly less accurate but still were reasonably precise (data not shown).

The efficacy of the BOTMAN treatment was examined in all 11 experiments and was compared with the untreated and weekly fungicide-only treatments. In all experiments, disease incidence in the BOTMAN treatment was significantly lower ($P < 0.05$) than the untreated control, and in all but one case, it did not differ significantly from that in the weekly fungicide treatment (Figs. 1 through 3 and Table 2). Disease reduction in the BOTMAN treatment averaged $63.9 \pm 3.0\%$ (mean of 20 categories plus/minus SE). This level of control was achieved with 2 to 7 fungicide sprays per experiment (mean of 4.2). In addition to the fungicide sprays, 4 to 9 *T. harzianum* T39 sprays were applied per experiment (mean of 5.9). In all experiments a spray was omitted only four times (mean of 0.4 sprays per experiment).

To identify the added contribution of *T. harzianum* T39 sprays beyond that achieved by fungicide sprays in BOTMAN, a treatment was included in all experiments whereby fungicide sprays were applied only when identical sprays were applied in the BOTMAN treatment. In some experiments, disease reduction achieved by this treatment was significantly less than that achieved by the BOTMAN treatment, indicating that the biocontrol agent improved disease control (e.g., stem infection, experiment C5; Table 2). However, in most experiments the disease reduction achieved by this treatment did not differ significantly ($P = 0.05$) from the BOTMAN treatment (e.g., experiment T1; Fig. 1A). Overall, average disease reduction in the fungicide according to BOTMAN treatment ($49.1 \pm 4.8\%$, mean of 20 categories plus/minus SE) was significantly less (paired *t* test, $P = 0.0012$; 19 degrees of freedom) than that in the BOTMAN treatment ($63.9 \pm 3.0\%$).

DISCUSSION

Biological control of plant pathogens is one of the means by which use of fungicides may be reduced. However, currently available biocontrol agents may not provide reliable disease suppression (11,12). In our study, for example, when *T. harzianum* T39 was applied alone, it was as effective as the fungicide in three of five experiments, effective but significantly inferior to the chemical fungicide in one experiment, and not effective in one experiment (Figs. 1 and 3 and Table 2). Consequently, biocontrol agents do not always meet commercial standards for disease management. Integration of chemical and biological control agents may provide an opportunity not only to reduce chemical use but also to cope more effectively with the development of pathogen populations resistant to common fungicides (11,12,17).

The components of the disease cycle of *B. cinerea* most sensitive to a fluctuating environment are spore germination and penetration. To germinate, spores of *B. cinerea* require a film of water for at least 7 h (14,35). In the Mediterranean basin, rain is the most

important weather parameter that contributes to the occurrence of free water on the foliage of plants grown in nonheated greenhouses. Consequently, epidemic outbreaks frequently are associated with rain events (Fig. 1A; [13]). The source of leaf wetness is not rain droplets, because the plants are protected from direct exposure to the rain but is instead condensation of dew. In non-heated greenhouses, the side vents are opened daily to permit air exchange; however, during rain events growers avoid opening the vents, the RH within the greenhouse increases, and the differences between air and leaf temperature result in the formation of a film of water on plant organs. Moreover, condensation of water on the inner side of the polyethylene covers drops on the plants, adding to foliage wetness.

With respect to management, prevention of spore germination and penetration is the purpose of fungicide application (24). The biocontrol agent *T. harzianum* T39 also inhibits spore germination and penetration (10,37). Because of the differences in the ecological requirements for spore germination of *B. cinerea* (i.e., the presence of a film of free water for at least 7 h and temperatures from 9 to 21°C [36]) and the antagonistic activity of *T. harzianum* T39 (no free water, 80 to 97% RH, and temperatures from 20 to 26°C [13]), warning systems may serve as a tool for identifying the times at which biological control is expected to be most effective.

Our initial efforts were devoted to determining whether consideration of past weather, future weather, or both is most appropriate for integration of control measures. Numerous warning systems have been developed by plant pathologists in recent years for a variety of plant pathogens. Many systems are based on past weather for timing of fungicide sprays (e.g., Blitecast, and FAST [25]), including models for *B. cinerea* (3). Although such an approach has been effective, it is a postfactum warning that something happened rather than a prediction that something will happen (36). One concern with this approach is that it may require fungicide applications after infections are initiated (19), when many fungicides are ineffective. Few researchers have examined the feasibility of using weather forecasts in a predictive system (28,32,33). We found that considering future weather as a basis for integration of biological and chemical control measures was an effective approach (Fig. 1). This outcome was expected, because most of the chemical fungicides currently available for control of *B. cinerea* inhibit spore germination, and such fungicides must be applied before infection occurs.

A *B. cinerea* warning system based on future weather was introduced in this study (Table 1) and was used to characterize the favorability of future weather for disease development and served as the basis for determining which control method, chemical or biological, would be more appropriate given expected environmental conditions. The precision of a warning system based on future weather depends on two components: the accuracy of the weather forecast and the validity of the decision-making process on which the system is based. The accuracy of the weather forecast was evaluated by comparing the forecast with actual measurements. The forecasts provided by the Israel Weather Service were reasonably accurate (Table 3). The second component, validity of decision-making, was not tested directly in our study. Alternatively, we examined the accuracy of the control actions directed by the warning system.

The chosen control method (chemical or biological) as determined by BOTMAN generally was justified. Because the disease cycle of *B. cinerea* requires 5 to 7 days (20) and each disease assessment included newly infected plant organs, it was possible to identify the specific effect of each spray. For example, in the 1994 tomato experiment (T1), seven chemical sprays were applied in the BOTMAN treatment. Just after the second, third, fourth, and fifth sprays, there was a sharp increase in disease incidence in untreated plots but not in treated plots, which indicates that the chemical fungicides were applied correctly, as intended, just before weather events highly conducive to the pathogen. Similarly, the application

TABLE 3. Accuracy of 4-day rain forecasts provided by the Israeli Weather Forecast Service in 1996^y

Forecast sequence	Prediction of rain quantity ^z		Prediction of daily rain event			
	True	False	Occurrence		Nonoccurrence	
			True	False	True	False
First day	23/26	3/26	6/7	1/7	18/19	1/19
Second day	22/26	4/26	4/5	1/5	19/21	2/21
Third day	23/26	3/26	5/7	2/7	18/19	1/19
Fourth day	19/26	7/26	7/9	2/9	15/17	2/17

^y The number of times in which the forecast of rain quantity (millimeters per day) and the occurrence or nonoccurrence of rain events (>0.5 mm/day) was true or false out of the total number of daily predictions.

^z Six ranges of rain quantity (millimeters per day) were defined: 0 to 0.4, 0.5 to 5.0, 5.1 to 10.0, 10.1 to 20.0, 20.1 to 30.0, and >30.0.

of five of the six sprays of *T. harzianum* T39 in the BOTMAN treatment was justified, because after their application disease progressed (in the untreated plots) moderately. A 'no-treatment' recommendation was given twice, at the beginning of the season and at the end; both were justified. BOTMAN recommendations, however, were not justified in all cases. For example, after application of the first, sixth, and seventh fungicide sprays in the BOTMAN treatment, disease progressed moderately in the untreated plots, indicating that conditions were suboptimal for the pathogen; under such conditions, application of *T. harzianum* T39 may have been sufficient. After the first spray of *T. harzianum* T39 in the BOTMAN treatment, disease in the untreated plots did not develop at all, indicating that a 'no-treatment' recommendation would have been more appropriate (Fig. 1A). Analysis of disease progress curves in all experiments revealed that the decision to apply fungicides in the BOTMAN treatment was justified in 26 of 38 applications (68.4%), the decision to apply *T. harzianum* T39 was justified in 46 of 57 applications (80.7%), and the decision not to treat was justified in all 4 cases. Tomato experiments T3 and T4 were not included in this analysis because it was not possible to identify the specific effect of each spray.

Madden and Ellis (25) suggested that proper validation of a warning system should include comparison of the system-generated spray schedule with standard spray schedule(s) and the no-fungicide control. These outlines were followed in our study. In all 11 experiments, reduction of disease in plots treated according to BOTMAN decision-making rules did not differ significantly from that achieved in plots treated weekly with a fungicide (Figs. 1 through 3 and Table 2). In the BOTMAN treatment, however, the use of fungicide was reduced by 65% compared to the weekly fungicide application. The conclusion is that integration of chemical and biological controls according to the BOTMAN decision-making rules enabled us to minimize the number of fungicides sprays without increasing the risk of uncontrolled epidemics and without decreasing the quality of disease control.

In most experiments, *T. harzianum* T39 sprays applied in the BOTMAN treatment did not improve disease suppression significantly beyond the suppression achieved by chemical fungicide sprays. Thus, one may consider omitting these sprays and using the warning system only for timing fungicide applications. In our opinion, it is important to include *T. harzianum* T39 for several reasons. BOTMAN uses forecast weather as a basis for decision making. A forecast may be inaccurate, with a mistake in one of two directions. One possibility is that wet weather is forecast, but in reality, dry weather occurs. This happened in 1996 in 21.4% of the forecasts (Table 3). In this case, the grower applied a chemical fungicide that was unnecessary. The cost of this mistake was the actual cost of application, the hazard to the environment, and the risk of resistance developing in pathogen populations, but the crop was not put at risk.

The second possibility is that dry weather is forecast, but wet weather actually occurs. This happened in 1996 in 7.9% of the weather forecasts (Table 3). The grower who does not spray at all may put his crop at risk. However, applications of *T. harzianum* T39 in between the chemical sprays may prevent damage. In that sense, the *T. harzianum* T39 sprays may be regarded as an insurance step taken by the grower. Integration of chemical and biological control methods is an important component of an antiresistance strategy (11,13). Application of biocontrol agents between chemical sprays may contribute to the prevention of resistance development toward chemical fungicides in populations of *B. cinerea*. It is possible that resistance to chemical fungicides already has developed in certain greenhouses but that the growers are not aware of it. In such cases, the biocontrol preparation can contribute to preventing control failure, because it will be applied in addition to the chemical fungicides. Finally, the *T. harzianum* T39 sprays did contribute to disease suppression in some experiments, and the mean disease reduction in the BOTMAN treatment (63.9 ±

3.0%) was significantly higher than that observed in the experiments using chemicals according to the BOTMAN treatment (49.1 ± 4.8%).

Because the concepts underlying BOTMAN were corroborated in the experiments, a procedure for their further implementation needs to be developed. We intend to develop a computerized decision support system that can be operated independently by the end user (i.e., extension personnel and growers). Such systems have been developed and used successfully for several other pathosystems (30,36). Another important step will be the development of a similar disease-management system for other pathogens that threaten greenhouse-grown vegetables, including powdery mildew and early and late blights of tomato and powdery and downy mildews and white mold of cucumber.

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